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CRITICAL NOTES ON THE PRESENT STATUS OF THE LENS-PROBLEM.

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1. Introduction.

The present communication is an attempt to arrive at a common understanding of the nature of the difficulties that stand in the way of the solution of a problem to which for over a quarter of a century have been devoted the best efforts of a number of biological observers.

One phase of this problem, although at one time rather near solution, has since, owing to—apparently unavoidable—technical difficulties of the experimental method, become ever more complex. In the following it is my intention to show that this complexity is an artificial one and that it practically disappears on careful sifting of evidence. Owing to this seeming complexity, however, an originally correct interpretation has lately been giving way to one that not only is unwarranted, but has already occasioned even some very improbable and fruitless phylogenetic speculations (cf. for instance Becher, '12).

Anticipating to return to the subject of this communication in a more comprehensive publication in the near future I hope I may be pardoned for incompleteness in considering the pertinent literature at the present time. Only such contributions can here be considered, as from my point of view seem to have the most significant bearing on the problem.

2. The Origin of the Primary Lens—in Ontogeny.

As well known Spemann ('01, '03) was the first to furnish experimental evidence for the correctness of the opinion—advanced simultaneously also by Herbst ('01)—that the lens of the vertebrate eye is dependent in its origin and differentiation

upon the contact of the optic vesicle with the supra-ocular epidermis from which it arises. It will be recalled also that this view was contested by Mencl ('03, '08), King ('05) and Stockard ('10) and that Spemann himself has in later papers ('08, '12) modified his opinion and abandoned his generalizations. He now thinks that more extended experiments in which various methods were employed on several frog species prove that in some species the lens cannot develop if the optic vesicle fails to come into contact with the overlying epidermis while in one species the epidermis is capable of giving rise to lentiform bodies and even well-differentiated, unmistakable lenses without such contact.

Such conditions would, indeed, be so contrary to expectation that it well behooves us to inquire into the validity of Spemann's self-contesting evidence as well, as of the evidence brought forth by Mencl, King and Stockard for the independent development of the lens.

I have already (Werber '16*c*) pointed out some possible sources of error which would invalidate the conclusions of these authors and on re-reading the entire series of Spemann's interesting papers on the subject I have been struck by the complete absence of any real counter-evidence against the conclusions of his earlier ('01, '03) excellent work.

Spemann's first experiments were performed on *Rana fusca* and consisted in destroying, by pricking with a heated needle or the galvanocauter, the right (presumable) optic pit (foveola optica).[1] As a result he found that no lens was formed, if the entire optic pit had been destroyed or, if deeply buried remnants of the latter which developed into diminutive optic cups failed to reach the epidermis. If, however, such a rudimentary optic cup did come into contact with the epidermis, a lens developed secondarily from the latter.

I am inclined to think that these results practically solved the problem, and for this reason it may perhaps be regarded as unfortunate that Spemann's well-warranted generalizations have been the subject of ill-founded criticism by Mencl ('03). For while the latter was very aptly met by Spemann ('03), it ap-

[1] For a description of this earliest discernible eye primordium cf. Eycleshymer ('93 and '95) and Froriep ('06).

parently occasioned him to enter upon a great many experiments on the subject which, though beautifully conceived and skillfully executed, have, owing to the pitfalls of a treacherous material, yielded fallacious results.

The first experiments which have occasioned Spemann ('07) to modify his views were performed on *Rana esculenta*. They consisted in the excision of the "right anterior half of the brain primordium" from the wide open medullary plate.[1] As a result he observed that a lens has developed in spite of the absence of an eye on the side operated upon. Like results were obtained also when the optic pit was destroyed at the same stage of development with a heated needle. The results were apparently entirely independent of the method of operation. In both cases, however, they differed from those obtained in *Rana fusca*, in which, as we have seen, no lens was formed when the optic anlage was at this stage destroyed by pricking with a heated needle, while the excision experiment in this species, according to Spemann ('07) "ergab ein sehr unsicheres Resultat." In one out of four examined embryos Spemann ('12) observed "ein kleines Bläschen, welches eine Linsenanlage sein könnte."

Why such divergent results in experiments on two species of the same frog genus with the same methods and at the same stage of development? Spemann concludes from his experiments on *Rana esculenta* that in this species the "lens-forming cells" of the embryonic epidermis are capable of independent development in the absence of an optic vesicle, while *Rana fusca* lacks this ability for self-differentiation.

The same experiments on *Bombinator pachypus* (at the same stage of development) gave less definite results. While no lens developed in this species on the side operated upon, Spemann observed in some few instances structures which he could not with certainty identify. Experiments on embryos of the same species at a later stage of development, *i. e.*, removal of the optic vesicle, the overlying epidermis being previously raised up and reflected from it, gave also ill-pronounced results. In a number

[1] In my 1916c paper several, otherwise wholly insignificant, errors have unfortunately crept in into the references made to these excision experiments in *Rana esculenta* and to King's pricking experiments. In both cases it should read "optic pit" or "optic anlage" instead of "optic vesicle."

of instances Spemann observed in such embryos on the side operated upon a thickening of the epidermis in the region where a lens normally should have developed, which he is inclined to consider as an abortive attempt at lens-formation. From these experiments Spemann tentatively concludes that *Bombinator* in regard to the power of independent lens-formation occupies a sort of intermediate position between *Rana fusca* which lacks this ability and *Rana esculenta* which, according to him, possesses it in a decidedly marked degree.

Are these conclusions justified? I am strongly inclined to think that they are not, the results on which they are based being very inconclusive. Moreover, on careful examination of the excision experiments in *Rana esculenta* the impression is gained that some fragments of optic substance were left after the excision of the anterior half of the primordium of the brain hemisphere. Thus, for instance, in his 1912 paper in Fig. 6 the smaller one of the two observed lentoids is in close apposition to a "ganglion." May not this ganglion rather be a small group of retinal cells? In Figs. 8 and 9 small groups of tapetum nigrum cells may be observed, and Spemann (p. 22) admits "dass vom Augenbecher vielleicht kleine Reste vorhanden seien." In Fig. 8 the tapetum-cells are very close to the lentoid, while Fig. 7 also suggests that dispersed tapetum nigrum was responsible for the origin of its "Linsenbläschen." Instead of concluding, as does Spemann, that the lentoids of Figs. 6 and 9 are due to dispersion of "lens-forming cells" it might perhaps be safer to think that in these experiments fragments of potential optic-cup substance were dispersed and stimulated the differentiation of lentoids by chancing to come into contact with the epidermis.

Again, in Fig. 10 representing a cross-section through an embryo in which the anterior part of the anlage of the right brain hemisphere has been destroyed by a heated needle, the lentoid observed is in close proximity to a rudimentary optic cup (oc′, Fig. 10), which clearly demonstrates the fact that the attempted elimination of the anterior part of the brain primordium may often be illusory, fragments of optic-pit substance remaining after the operation. According to Spemann ('12, pp. 24 and 25) it is easier to eliminate the entire optic anlage in *Rana fusca* than

in *R. esculenta.* He attributes this to a difference in the "Konsistenz" between the embryos of the two species. "So ist wohl das Resultat zu erklären, dass der Defekt bei den allerdings nicht sehr zahlreichen Versuchen dieser Art meist entweder zu gross wurde, im ersteren Fall also vom rechten Auge ein Rest erhalten blieb, im letzteren die Linsenanlage mit zerstört wurde."

In my estimation the divergent results obtained both by pricking with a heated needle and by excision in both species would rather seem to point to differences in the location of the optic pits in the neurulae of the two species at the same stage of development. Thus, in *Rana esculenta* they are probably more diffuse and extend more laterally than they are in *R. fusca.* Accordingly, if at this stage the excision of the anterior half of the brain primordium be attempted, it may happen that a small fragment of the optic pit may remain, as in this species it may extend even laterally from the medullary fold. Granting, however, that fragments of potential optic-cup substance do sometimes remain after the operation in this species—and Spemann admits that both in text and in the figures—it is no longer difficult to understand that in such embryos lentoid structures and even well-differentiated lenses may be formed on the eyeless side owing to the "lentogenic stimulus"[1] from the remnant of optic substance on the epidermis.

Spemann does not seem to have considered the results obtained from these operations as very conclusive, if as much can remain of the brain anlage after its attempted elimination as "ein Fragment des Tapetum nigrum," "der vorderste Teil der Vorderhirnanlage, . . . ein dorsales Stückchen Zwischenhirn mit Epiphyse und Plexus chorioideus, jedenfalls entstanden aus lateralen Partien der Medullarplatte, endlich ein Stückchen der linken Hälfte des Mittelhirns" ('12 p. 28). For, not content with these results he sought confirmation in experiments in which several other methods were employed.

Thus, adopting Lewis's ('04) method, he removed (by excision) in *R. esculenta* the optic vesicle after having previously raised up and reflected the overlying epidermis. The latter was then affixed in its place where it healed and in two embryos gave rise

[1] Cf. Werber '16c.

to "ein deutliches Linsenbläschen" in the absence of the optic cup. Spemann considers these lens-buds as evidence of the ability for independent lens formation in *R. esculenta.*

The validity of this conclusion, however, seems questionable to me in view of a well-nigh uncontrollable source of error which Spemann himself has pointed out ('12, p. 42). For he states expressly that the lower stratum of the epidermis (the "Sinnesschicht") adheres so firmly to the eye vesicle that on attempting to separate the epidermis from the latter usually the upper layer (the "Deckschicht") is raised up, while the lower one remains attached to the optic vesicle. Regardless of Spemann's skill in such delicate operations and the precautions he has taken to separate the entire epidermis from the optic vesicle it was apparently impossible always to avoid minute fragments of the latter remaining attached to it. And the two cases in which he obtained "independent" lens-buds have possibly resulted from just such an unsuccessful operation, while in the embryos in which no such structures were recorded on the side operated upon the operation was apparently faultless.

Spemann assumes that in his unsuccessful experiments of this series, *i. e.*, where no "independent lenses" were recorded, the "lens-forming cells" of the lower stratum of the epidermis were removed with the optic vesicle to which they remained attached. I can see no valid reason for the assumption of cells predetermined to form lenses. The often-raised argument of the cyclopean eye in which the lens is formed from epidermis that normally does not develop into this structure, and Lewis's ('04, '07a and b) experiments in which the formation of lenses from strange ectoderm and even from ectoderm of another species was demonstrated to be possible—an experiment successfully repeated by Spemann himself by transplanting the bared optic vesicle of *Rana esculenta* under the ventro-abdominal epidermis of *Bombinator*—speak very decidedly against this assumption.[1]

Moreover, why should just the species *Rana esculenta* form such a strange exception as to possess such so early specialized

[1] Mencl ('08, Fig. 3) described an "intracerebral eye" (intracerebral cyclopia) whose lens is derived from the epithelium of the mouth. The ability of this part of the ectoderm to give the "lentogenic reaction" is demonstrated also by the lentoids of the mouth which I have recorded (Werber '16c).

regions of the epidermis? I am under the impression that the apparent fallacy of Spemann's conclusions from this series of experiments is most likely due to the circumstance that while he detected an important source of error, he unfortunately, mistook the nature of the error. For had he considered the possibility that fragments of the optic vesicle too small to be detected with the binocular dissecting microscope and even so minute as not to be able to differentiate into histologically discernible structures, may suffice to induce the lentogenic reaction in the epidermis to which they remained attached, he would probably have considered the "unsuccessful" experiments of this series as successful and vice versa. Accordingly, instead of believing that in the two "successful" cases the "lens-forming cells" had not (or not entirely) been removed, he might have concluded that owing to the above-mentioned difficulty some remnants of optic substance have stimulated the development of the lens-buds demonstrated in Figs. 37, 38 and 38a.

The results of other experiments performed by Spemann ('12) which may now be examined would also seem to lend support to his former views rather than to his present ones. One of these experiments consisted in transplanting abdominal epidermis over the bared optic vesicle in *Rana esculenta*. The flap of epidermis used for the purpose was previous to its transplantation "von etwa anhaftenden Mesodermzellen sorgfältig gereinigt." The result was negative—no lenses or lens-like structures were formed by the strange epidermis owing, as Spemann thinks, to the circumstance that the "primären Linsenbildungszellen" were contained in the flap of supra-ocular epidermis which had been removed. However, four cases were recorded in which the epidermis transplanted over the eye presented an appearance different from that of the epidermis of the immediate surroundings. And in Fig. 63 there is "eine deutliche Linsenanlage (L) vorhanden, ein kleines, dickwandiges Epithelbläschen, welches mit der transplantierten Rumpfhaut über dem Auge innig verbunden ist." ('12, p. 55). Spemann mentions also another embryo of this series in which he observed an indication of lens-formation on the side operated upon (Fig. 58), but assumes that in both cases "primäre Linsenbildungszellen" remained attached

to the optic vesicle owing to faulty technique in the removal of the supra-ocular epidermis.

Might we not with at least just as much justification assume that in the embryos in which no lenses were formed from the transplanted epidermis this was due to lack of immediate contact between the optic vesicle and the epidermis, because mesodermal cells were attached to the latter? For, regardless of the most painstaking care I think it is impossible to be sure that the epidermis has been cleaned of these cells entirely with the aid of such low magnifications as even the highest power lenses of the binocular dissecting microscope. It is, besides, difficult to understand why the optic vesicle of this species should be able to induce lens-formation from ventro-abdominal epidermis of *Bombinator* and not from the abdominal epidermis of an embryo of its own species.

Whatever one might think of the results of these experiments, they certainly cannot be regarded as conclusive in favor of Spemann's present views.

Two more series of experiments on *Rana esculenta* may now be considered the results of which Spemann interprets in favor of the independent development of the lens in this species. The method employed in these experiments was autoplastic transplantation and the part transplanted was the "primären Linsenbildungszellen."

In the first (a) series a more or less rectangular piece of supra-ocular epidermis was detached during or immediately after the closure of the medullary tube and turned about 180°, care being taken that no fragments of the optic vesicle be left on it. In the second (b) series the operation differed only in one point, viz., that in detaching the supra-ocular epidermis a fragment of the optic vesicle remained attached to it and owing to the inversion of the piece of epidermis (by turning it about 180°), it became transplanted into a posterior region (otic capsule).

In the experiments of the first (a) series great technical difficulty was encountered and only in four cases was the separation of the epidermis from the optic vesicle "anscheinend einwandsfrei." In three of these cases the eye of the operated side had "eine deutliche Linse," while in the fourth case where, owing to

the operation, the otic labyrinth came to lie between the epidermis and the eye, the latter lacked a lens. No lens, however, developed in any of these embryos caudally from the eye, as might have been expected, had that part of the epidermis ("primäre Linsenbildungszellen") been capable of giving rise to a lens by self-differentiation.

These cases (although so few in number) point decidedly, I think, to the inability of supra-ocular epidermis in *R. esculenta* to differentiate a lens in the absence of a stimulus from the eye vesicle. Spemann, however, prefers the tentative explanation "dass nach Auslösung einer Linsenbildung durch's Auge die spontane Entwicklung einer zweiten Linse aus den primären Linsenbildungzsellen unterbleibt." He is inclined to consider even the possibility "dass bei diesen Experimenten die Linsenbildungszellen nicht weit genug nach hinten gebracht worden waren und dass entweder sie selbst oder ihre nächste Umgebung die Linse des stehengebliebenen Auges geliefert haben." As support for the latter possibility he regards the observation, "dass die Linse in allen 3 Fällen im hintersten Winkel des etwas deformierten Augenbechers liegt und einmal sogar etwas in die Länge gezogen ist" ('12, p. 64). Is it not more probable that this distorted relation between the lens and the optic cup is due to the very fact that the latter was deformed owing to the operation? From my observations on many teratophthalmic *Fundulus* embryos I know that this is very often the case in deformed eyes.

One cannot escape the impression that Spemann by considering such possibilities and by regarding "diese Versuche . . . als misslungen und die Frage als unentschieden" has—apparently unconsciously—missed the only obvious conclusion, viz., that *Rana esculenta* possesses no early predetermined lens-forming cells and that a stimulus from an optic cup or from some of its parts is necessary to induce the differentiation of a lens from the epidermis in embryos of this species as much as in *Rana fusca*.

Let us now consider the second (b) series of these experiments in which, as will be recalled, a fragment (the tip, "Kuppe") of the optic vesicle was left attached to the inverted epidermis.

Observations were made on seven embryos operated upon in

this manner. In all of them the anterior eye fragment lacks the lens, while the posterior one in five embryos stimulated the formation of a lens which "*kann* so vollkommen entwickelt sein wie die normale" (p. 67). In one of these embryos the posterior eye fragment possesses even two lenses which Spemann is inclined to regard as being due to mechanical separation of the "Linsenmaterial" into two parts, although it would seem more probable that the duplication of the lens in this case may (owing to the operation) be due to mechanical histolysis of the eye fragment which thus came into contact with the overlying epidermis in two places.

From these observations Spemann (p. 67) concludes that "die weitgehende Determination der primären Linsenbildungszellen von *Rana esculenta*, welche sie zu selbstständiger Entwicklung befähigt, bringt es wohl mit sich, dass die übrigen Epithelzellen, auch die der nächsten Umgebung, nicht mehr imstande sind, auf einen Reiz des Augenbechers mit Linsenbildung zu antworten."

These conclusions cannot be considered as warrantable. Moreover, the results permit of an interpretation which, I believe, is founded upon a much greater probability. It must be remembered that at the stage of development at which the operations were performed, it is only the tip ("die Kuppe") of the eye vesicle that is protruding from the head. If that protruding part be removed, the epidermis transplanted over the resulting gap may, owing to the latter, fail to come into contact with the deeper part of the eye even on subsequent growth of the latter owing to the obstruction formed by mesenchyme cells which may grow into the gap before such contact can be effected. Spemann's Fig. 67*b* very forcibly suggests just this possibility. On examining the anterior eye fragment of this figure one can observe that it is not in contact with the epidermis. More significant, however, is the observation in its lumen of a blood vessel and of some mesenchyme cells which also fill the space between it and the epidermis.

Our interpretation of these apparently vexing cases receives further support from the very fact that in the (a) series of these experiments where the "Kuppe" of the optic vesicle had not

been removed, the latter was able to stimulate the formation of lenses, because there being no gap between it and the epidermis transplanted over it, there was no chance for an obstruction of its contact with the latter by ingrowing mesenchyme. It is, besides, needless to say that it is difficult to understand just why Spemann in the experiments in which the epidermis was transplanted over a defective eye vesicle assumes a sharp predetermination to form lenses and not so, if transplanted over an uninjured eye vesicle.

Summarizing briefly *we find that critical sifting of the results of Spemann's experiments on Rana esculenta discloses no warrantable evidence whatsoever for the presence in this frog species of an early predetermined lens-forming part of the epidermis capable of differentiation into a lens without the stimulus from an optic vesicle or at least a fragment of it.*

Practically all of these experiments were performed by Spemann also on *Bombinator pachypus.* The results obtained in this species differed according to Spemann ('07, '12 ff) from those obtained in the pricking experiments in *Rana fusca* and also from the above-noted results in *Rana esculenta.* They indicate, Spemann believes, that *Bombinator* in regard to the ability to form a lens by self-differentiation occupies a position intermediate between *Rana fusca* and *Rana esculenta.*

It is evident, I believe, that were this really so, *i. e.*, if the supra-ocular epidermis of *Bombinator* possessed a certain degree of this ability and only needed the stimulus from the optic vesicle as a complementary aid for the differentiation of a lens, a serious stumbling block would here confront every attempt at the solution of the lens-problem.

Fortunately, however, Spemann's observations seem to call for an entirely different interpretation of the results.

Thus in the excision experiment (right foveola optica) the latter prove very decidedly, I believe, that no lens can be formed in the absence of an optic anlage or on its failure to come tinto contact with the overlying epidermis. For, out of the forty-six embryos operated upon in twenty of them in which an optic fragment remained (owing to incomplete excision), a lens developed on the side of operation. Of the remaining twenty-six

cases one is uncertain, for here a structure was observed which might perhaps be a deformed lens-bud, in twenty cases neither an optic cup nor a lens can be observed, while in five cases the optic cup is so small that there was no contact with the epidermis. According to Spemann, however, in two of the latter cases the condition is not clear, one of the embryos possessing (on the side of excision) near the brain "ein dickwandiges Bläschen mit kleinem Lumen," which Spemann is unable to identify, while in the other case the region of epidermis which should have furnished the lens, is "kaum merklich verdickt."

The only warranted conclusion from these results is: *The contact of at least a fragment of the optic anlage is necessary to induce the formation of a lens from the overlying epidermis.* It is unwarranted however, to conclude, as does Spemann (12, p. 38), ". . . dass Bombinator pachypus zwar auch primäre Linsenbildungszellen besitzt, welche von den Epidermiszellen der Umgebung verschieden und zur Umbildung in die Linse vorbereitet sind, dass diese Zellen aber der Mitwirkung des Augenbechers bedürfen, um in Aktion zu treten, zum mindesten in viel höherem Masse als die Linsenbildungszellen von R. esculenta."

The results of other experiments in this species also point decidedly to the incorrectness of Spemann's conclusions. Thus the removal of the optic vesicle during or immediately after the closure of the medullary folds resulted only in a thickening ("Wucherung") of the epithelium in the region where normally the lens should have arisen. In two cases, however, small remnants of the optic vesicle developed into diminutive optic cups lacking a lens, because mesenchyme had grown in between them and the epidermis. Lentoid structures (two) were observed only in one case (Figs. 41*a* and *b*, L′), which are probably due to a stimulus from very minute remnants of the optic vesicle.

These experiments would again seem to *prove only that,* as Lewis ('04, '07*a* and *b*) and Le Cron ('07) have found, *a stimulus from the optic vesicle on the epidermis of sufficient duration is necessary for the latter to give rise to a lens.* They do not prove, however, the presence in this species of early predetermined lens-forming cells requiring the stimulus from an optic vesicle only as a supplement for full differentiation into a lens.

On transplanting ventro-abdominal epidermis over the bared optic vesicle in this species Spemann recorded as a result that in none of the sixteen examined embryos a lens developed from the strange epidermis. From these results he concludes that either the optic vesicle cannot stimulate abdominal epidermis to the formation of a lens or that this part of the epidermis is incapable of responding to such a stimulus.

Here again, however, the same objection is unavoidable which we have raised against the conclusion from the results of the same experiments in *Rana esculenta*, namely that regardless of the experimenter's care mesenchyme cells attached to the transplanted epidermis obstructed a contact of the latter with the optic vesicle.[1]

Very clear ("eindeutig") results were obtained in the experiments in which, as in *Rana esculenta*, a flap of the skin of the head including the supra-ocular epidermis was turned about 180°. In the first (a) series in four out of eight "einwandsfreie" cases the eye cup was not in contact with the epidermis and, naturally, lacked a lens. In the other four cases there was such contact—and the eye possessed a lens.

Accordingly: *There are no lens-forming cells, but epidermis that normally does not give rise to a lens will differentiate into a lens, if brought into contact with the optic vesicle.*

The second (b) series of these experiments (a fragment, the "Kuppe," of the optic vesicle transplanted with the turned epidermis) yielded results very similar to those of the corresponding experiments in *Rana esculenta*.

With but one exception the posterior eye fragment always obtained a lens, which, as Spemann rightly adds, could not have developed in the absence of this fragment of optic-cup substance.

The anterior eye fragment, however, obtained no lens in fifteen out of the twenty examined embryos, while a lens or only a thickening of the epidermis opposite the eye fragment was ob-

[1] It is significant that in one out of the four cases in which he reports to have observed a "Wucherung" opposite the eye "eine zusammenhängende Schicht sehr dotterreicher Zellen" (p. 60) was conspicuous in that interspace. That shows that it is apparently futile to attempt to clean the abdominal epidermis of all mesenchyme cells. While it is difficult to know just how to interpret these "Wucherungen," they might possibly be due to mechanical distortion during the operation.

served in five cases. In thirteen of the embryos which had no anterior lens Spemann accounts for its lack by the ingrowth of mesenchyme into the space between the optic-vesicle fragment and the epidermis, widened by the expansive growth of the otic capsule which, owing to the transplantation came to occupy this anterior position. He is unable, however, to suggest an explanation for the lack of this lens in two cases, "wo kein Grund für ihr Ausbleiben zu erkennen ist" (p. 73).

From these experiments Spemann ('12, p. 77) concludes that the optic vesicle of *Bombinator* is capable of exerting a specific stimulus not only on the lens-forming cells, but also on other parts of the head epidermis.

This conclusion is, I think, only partly correct, the assumption of "primäre Linsenbildungszellen" being both unwarranted and unnecessary. For leaving out of consideration the two cases (which I should not wish to pre-judge), where no anterior lens was formed while it should have been expected, we can conclude only that its presence in the several other cases was due to the specific stimulus from the anterior eye fragment.

Briefly, the results of this series of experiments also contradict Spemann's opinion of the intermediate position of *Bombinator* between *Rana fusca* and *R. esculenta* with regard to the ability of independent differentiation of the lens.

In *all of Spemann's work* I find only *a confirmation of his initial results and strong support for the generalizations which he made in 1901 and 1903, but no counter-evidence whatsoever that would justify his present ('12) opinion.*

Other evidence for the independent development of the lens brought forth by Mencl ('03, '08), King ('05) and Stockard ('10) is no less illusory.

Mencl ('03) described the head of an anophthalmic component of an anadidymus in *Salmo salar* which possessed two laterally located lenses although there could be observed "keine Spuren von Augenblasen, ja nicht einmal von Anlagen derselben." Both of these lenses were in close apposition to the deformed brain, the larger one even having, owing to its growth, formed a pit in the latter by pressure. Both lenses, however, were so situated that their derivation from lateral parts of the head

epidermis was beyond doubt. What is responsible for the origin of these lenses? Mencl suggested (p. 337) that "der, diese zwecklose, wie durch Erinnerung der Epidermiszellen auftauchende Linsenbildung auslösende Faktor ist die Vererbung."

A much more reasonable and, I think, the only correct, interpretation of this case was offered by Spemann ('03) who concluded (p. 464) that the optic vesicles "oder genauer ihr für die Linsenbildung allein in Betracht kommender retinaler Teil nur scheinbar fehlen, indem die Partie der Hirhwand, welcher die Linsen angelagert sind, nichts anderes ist, als die nicht abgegliederte . . . Retina."

Although this interpretation was later abandoned by Spemann "unter dem Druck neuer Tatsachen" ('12, p. 3), my experience with teratological material—and Mencl's case is a teratological one—forces me to lend it unreserved support.

For the sake of clearness in presenting my point of view (based largely on teratological data) the following brief recapitulation of the present stage of our knowledge on the morphogenesis of monsters may be permitted.

It was repeatedly noted by pathologists and teratologists and notably also by Mall ('09) that examination of malformed embryos often discloses evidence of destruction, dissociation and shifting of tissues or parts of the embryo. On examination of a great many experimentally produced monsters in *Fundulus heteroclitus* I ('16*a* and *b*) was able not only to confirm these observations, but also to demonstrate some remarkably striking cases of the effects of such dissociation of parts of the early embryonic primordium and to account for the causes of this process which I have termed blastolysis. This "blastolytic action of the chemically modified environment is . . . a morphogenetic principle common to all terata. . . . Blastolysis either destroys part or all of the germ's substance, or it may split off and disperse parts of the latter" (Werber '16*b*, p. 569).

It is this destruction of tissues, the subsequent elimination of parts destroyed and the resulting dissociation and shifting of parts surviving that in experiments in which eggs are subjected to a chemical modification of the environment, brings about the weirdest malformations of the developing embryos.

All of these deformities are thus clearly *due to a defect* (*or defects*) *of a blastolytic nature.* Not only is this the case in experiments in which the modification of the environment employed is a chemical one, but also, if a physical, *e. g.*, thermic, modification be employed. For, in experiments (not yet published), in which the eggs of *Fundulus heteroclitus* were subjected to the action of a temperature much below the normal, some terata and particularly ophthalmic monsters resulted. The latter are due to loss at an early embryonic stage of parts of ophthalmoblastic material and to like damage sustained by the anterior part of the potential head. Thus anophthalmia results from such loss by the earliest brain primordium of the whole or nearly whole ophthalmoblastic material.

In some anophthalmic embryos such blastolytic optic-cup fragments or even small, fairly well differentiated optic-cups may on microscopic examination of sections be observed either enclosed in the brain between both hemispheres (intracerebral cyclopia, as it were, cf. Mencl '08, Figs. 2 and 3) or in a lateral position as a part of the brain. Such "concealed" remnants of the optic cup may often lack the tapetum nigrum and the histological character of retina, owing to early destruction of some groups of cells which potentially corresponded to the lacking layers of the retina, and may thus give the appearance of a part of the brain. If it happens to come into contact with the epidermis, such "a part of the brain" will stimulate the differentiation of a lens from it.

This, undoubtedly, is exactly the condition in the case described by Mencl in 1903. I have many times observed unmistakable optic cups which lacked some of the histological characteristics of retina and whose structure was very much like that part of the "brain" in Mencl's case (cf. Mencl '03, Figs. 2, 4 and 5, and also '08, Fig. 1), into which the larger one of the two lenses has, as it were, burrowed in. That part of the "brain" is undoubtedly a "verkappte" retina, as Spemann had once suggested.

This, I think, disposes of the first, but most troublesome, case described by Mencl. It might perhaps be added that the asymmetric position of the two lenses[1] in the head of this anophthal-

[1] "Die linke liegt mehr dorsal und zugleich caudalwärts von der rechten" (Mencl, '03, p. 331).

mic anadidymus component could not be accounted for by "hereditary reminiscences," while chance contact of remnants of dissociated ophthalmoblastic material of both sides with the epidermis does account for the origin of both lenses as well as for their asymmetrical position. Neither of them has arisen from predetermined, lens-forming cells—"durch Erinnerung," but from indifferent ectoderm in response to stimuli from remnants of potential optic cups on the epidermis.

The other "independent" lenses and lentoids described by Mencl ('08) can even much easier be demonstrated to be due to the same origin—blastolysis and subsequent contact of fragments of optic cup substance with the ectoderm. From his description of sections of the embryos underlying the Figs. 4, 5, 6, 7 and 8 the reader will easily gather that in these cases fragments of optic-cup substance[1] have been profusely dispersed and in places of contact with ectoderm gave rise to lenses or lentoids. Mencl did not appreciate the full significance of his own observations, for referring to such an optic fragment ("Pigmentanhäufung," Fig. 5*X*, he says: "Was dies Gebilde bedeutet, *kann ich nicht entscheiden—das Vorhandensein des Pigments jedoch, sowie die retinaartige Anordnung der Zellen in der benachbarten Hirnwand*[2] lässt die Meinung entstehen, dass es sich dabei um irgend ein Rudiment einer atypisch und selbstständig zur Entwicklung gelangten Augenkomponente handelt" (p. 447). It is, indeed, difficult to understand just why, in spite of these observations, Mencl claims that in these embryos "die selbstständige Entwicklung der Linsen über jeden Zweifel erhaben ist" (p. 447).

I have recently examined sections of a number of deformed Salmonid embryos (of another species) with such "independent" lenses. The publication of the observations made on this material being reserved for a future publication, it may suffice to state that the conditions noted in several of these embryos bore a very striking resemblance to those in the embryos lately described by Mencl ('08). Here, too, subjective interpretation

[1] "Pigmentanhäufung," "Pigmentbläschen," "Pigmentfleck," "eine homogene, schwarze, längliche Pigmentmasse, welche in die Falte der retina-artigen Hirnwand hineinragt" (Mencl '08, p. 447).

[2] My own italics.

might easily lead to the conclusion that the "eyeless" lenses arose by self-differentiation of the ectoderm. But careful observation and rigid analysis of the noted relations of parts to each other leaves no doubt that all these lenses are products of stimuli from dispersed optic-cup substance.

In a *Fundulus* monster described by me in a former paper (Werber '16*c*) there were no eye defects, but owing to a special method optic-cup substance was very profusely dissociated and dispersed through a large part of the head. Owing to this condition a great deal of the head ectoderm and even the epithelium of the mouth, infected, as it were, with such fragments of the optic anlage, responded by the formation of a great number of lentoids. Many more monsters resulting from the employment of the same method were examined in sections and they show very similar conditions. In other experiments in which the method employed was more destructive various eye defects such as monophthalmia or synophthalmia or anophthalmia resulted. On examination of sections through these embryos not only lentoids, but well-differentiated, "eyeless" lenses were frequently observed and in nearly every one of these cases some more or less obvious traces of optic-cup substance can be observed in their immediate neighborhood.

In the same paper I have pointed out that the "independent" lenses which Stockard ('09, '10) described in teratophthalmic *Fundulus* embryos have also undoubtedly resulted from contact of ectoderm with such blastolytic fragments of the eye anlage.

On that occasion I have also called attention to a very probable source of error in King's ('05)[1] experiments in view of which the evidence she brought forth for the independent development of the lens in *Rana palustris* in contradiction to Lewis's ('04) unmistakable evidence to the contrary, appears to be illusory.

Experiments of other observers (Bell '06 and '07 and Ekman '14) also yielded results which decidedly contradict the idea of the independent development of the lens. And a very beautiful demonstration of the "lentogenic reaction" of the epidermis to a stimulus from an eye fragment was recently furnished by Wachs ('14, p. 430 and Figs. 46, 47, 48 and 49).

[1] Cf. the footnote on p. 221 of this paper.

This author transplanted in well-advanced larvæ (hind legs developed) of *Triton tæniatus*, in which the eye was at the time of operation as fully developed as in the adult, a piece of the iris from the eye of one larva into the otic capsule of another larva of the same age. As a result he could in one case observe that a strand of epidermal epithelium which, owing to the operation, had grown in from the edge of the wound to the otic capsule, has given rise to "ein rundes Lentoid . . . mit konzentrisch gelagerten Linsenfasern . . .."

From all that has been said so far we may conclude that *there exists no valid evidence for the possibility of the origin of the lens by self-differentiation.* On the contrary, *observation in the normal development of the eye, evidence from teratophthalmic cases and all experimental evidence point to the correctness of Herbst's ('01) and Spemann's ('01) conclusion that the lens of the vertebrate eye depends in its development and differentiation from ectodermal epithelium upon a specific, apparently chemical, stimulus from the optic vesicle.*

This stimulus might perhaps be in the nature of an enzyme action by contact. Spemann ('05, '12), who recognizes the dependence of the development of the lens upon this specific stimulus in some species, assumes the possibility of a specific secretion by the optic cup or rather by the retinal part of the latter, to which, as he believes, may also be due the "regeneration" of the lens from the iris of the fully developed amphibian eye. While this hypothesis appears to have received strong support from the beautiful experiments of his pupil Wachs (*l. c.*), it seems to me perhaps premature to speak of a secretion in this case. For, aside from other considerations, the fact ascertained by Wachs (*l. c.*) that the supposedly secreted substance is not conveyed by the blood of the animal to any other part of the body would seem sufficient to indicate that the retina, as Wachs himself concludes, has no such endocrine function. Nor is Wachs's assumption of a secretion that "bleibt auf das Auge und seine nächste Umgebung beschränkt" (p. 446) justified by the results of his numerous experiments.

There is, moreover, good reason to believe that the capacity for the "lentogenic stimulus" is not at all restricted to the retinal

layers of the eye cup. For evidence is not lacking that the tapetum nigrum is also capable of inducing the development of a lens (or lentoid) when in contact with ectodermal epithelium. Thus, as pointed out above (p. 235), some of the "independent" lenses recorded by Mencl ('08) very clearly owe their origin to contact of ectoderm with tapetum nigrum fragments ("Pigmentbläschen," "Pigmentanhäufungen." "Pigmentmasse"). It was also mentioned that in a considerable number of *Fundulus* monsters in my possession "independent" lenses and lentoids can be demonstrated to be due to this "enzyme action" of the tapetum nigrum. This ability of the pigment layer of the optic cup is also suggested by some of Spemann's ('12) and Stockard's (*l. c.*) figures.

Whatever the nature of this action may be, whether or not we agree to regard it as an enzyme action, as I am inclined to do, it seems evident that all layers of the optic cup are capable of it. Even the iris epithelium which genetically is also a part of the optic cup may also be capable of this action.

3. The Origin of the Secondary Lens—in "Regeneration."[1]

Bearing in mind this apparent ability of the embryonic ectoderm for lens formation—by "enzyme action," it may perhaps no longer be difficult to account, at least theoretically, for its secondary formation—"regeneration"—from the iris after the exstirpation of the primary lens from the fully developed eye of the amphibian larva or even the adult.

An attempt in this direction has already been made by Wachs (*l. c.*) who accounts for this phenomenon as a reaction of the epithelium of the iris to the "secretion" of the retina. Although I find it difficult to accept the evidence for this secretion, I believe that Wachs's idea is correct in the main. The secondary for-

[1] I am not inclined to regard the formation of the lens from the epithelium of the iris as a case of true regeneration in the precise meaning of that term. *From the histogenetic point of view* we must say that *this matrix* (*the iris*) *gives rise to the lens for the first time* and thus it *generates*, but does *not re*generate, it. *Considering*, on the other hand, the *secondary lens in relation to the whole visual organ or to the entire body of the animal*, it is difficult to deny that *we are here dealing with* a case of what is commonly regarded as *regeneration* ("Ersatz", "Nachbildung", "Nachwachsen", "Wiederwachstum"). Without wishing, therefore, to decide on the fitness of the term in this connection, I am employing it only in quotation marks.

mation of the lens—from the epithelium of the iris—is probably a response (reaction or complex of reactions) to a chemical stimulus just as much as its primary development from the supra-ocular epidermis of the early embryo.

But, it may well be asked, if there is no such secretion as assumed by Wachs, what is the nature of the chemical stimulus and whence, from what source, or from what part of the eye does it issue? Two possibilities suggest themselves as an answer to this query. It may be imagined that, owing to the operation, the iris comes into temporary contact with the inner wall of the optic cup, from which it might thus receive the stimulus for the formation of a lens in the same manner as in the embryo the supra-ocular epidermis from the optic vesicle. Such contact due to a collapse of the optic cup[1] may result from the methods of operation employed by Wolff ('95) and the other experimenters (with the exception of Wachs). However, I am not inclined to give serious consideration to this possibility, as this contact of the pupillary edge of the iris is not necessary for the "regeneration" of a lens, for in Wachs's experiments the method of operation excludes it altogether. The experiments of the latter author, however, do not, as he believes, exclude another possibility which to me seems well worthy of careful consideration.

Wachs (*l. c.*, pp. 416–426) has raised the question whether the iris is capable of forming a lens without any outside stimulus ("aus sich heraus"). He was led to consider this possibility by the results of some of his experiments, in which a fragment of the iris, implanted into the posterior chamber of an eye deprived of its lens "regenerated" a lens, even if it did not heal on to the iris. This lens may even be better differentiated than the lens simultaneously "regenerated" by the latter.

To decide whether the fragment of iris in these cases formed a lens under the influence of a secretion from the retina or—"aus

[1] According to a statement by Müller ('96) no lens is formed by the iris after extraction of the primary lens, if the vitreous body is injured. Were this statement correct, no lens could arise from the iris owing to its contact with the optic cup by collapse of the latter. It is evident, however, that Müller's observation is incorrect, for in Wolff's (*l. c.*) and in some of Fischel's ('00 and '02) experiments in which a lens or lentoids did form from the iris, the vitreous body was undoubtedly injured, since the whole optic cup was damaged.

sich heraus," he transplanted (in a number of experiments) a fragment of the iris under the skin of the head. The result was negative, as the piece of iris disintegrated and was resorbed.

Wachs concluded from this result that the site of this transplantation was apparently unfavorable and he, therefore, in the next experiments transplanted a fragment of the iris into the otic labyrinth, after first removing a part of the latter. The following results were obtained:

In several cases where the fragment was "small," *i. e.*, consisted of iris only without retinal cells or with some very few of the latter, no lens-like structures were formed. In a number of instances, however, where the fragment was "larger," *i. e.*, containing more retinal cells, lentoids were formed (apparently from the iris), while in several other cases the transplanted fragment was transformed into a small eye with a lens, which latter Wachs considers as formed from the fragment of iris. However, in a number of instances (pp. 425, 426–428) where the transplanted fragment contained many retinal cells and was in good condition ("trotz guter Erhaltung") no lens-like structures were formed.

These results are, obviously, inconclusive. They certainly do not permit of Wachs's conclusion that the lentoids and lenses of the "positive" cases owe their origin to a stimulus from a secretion of the retinal cells (carried with the transplanted fragment of iris). For, why could not such effect of the retinal "secretion" be observed in the "negative" cases in spite of considerable retina? From these results I can read no definite, unmistakable, answer to the question which they were to answer.

For the following reasons, however, I should regard the query as a very pertinent one.

We know that the retina (Spemann, Lewis, Le Cron, Bell, Ekman, Werber and others) can furnish the stimulus for the formation of a lens from an epithelial derivative of the ectoderm (even epithelium of the mouth—Mencl, '08, and Werber, '16*c*) by contact with it. Besides being capable of furnishing the stimulus for this "lentogenic reaction" the retina (an ectodermal derivative) is according to Fischel's ('00, '02) and my (Werber, '16*c*) observations capable also of responding to such a stimulus—the lentoids of the retina. Whence does this stimulus issue in

the case of the formation of the retinal lentoids? Considering the fact that the retinal cells can both furnish the "lentogenic" stimulus and respond to it, it would certainly seem not far fetched to assume that the stimulus for the formation of the lentoids by the cells of the retina issues from themselves. In this case the retinal cells react to their own "lentogenic enzyme." Both the conditions for furnishing the stimulus and the latent potency of responding to such a stimulus are known to be present in the retina. May not this double capacity underly also the formation of the lens from the iris?

To answer the latter question it would be necessary to find out whether the iris besides its known potency of forming a lens (Wolff, Müller, Fischel, Wachs, and others) is also capable of furnishing the "lentogenic stimulus," if in contact with epithelium (or some other ectodermal derivative). Unfortunately, however, the question cannot be answered definitely at the present time.

There exists only one experiment (by Wachs, *l. c.*, p. 430 and Figs. 46, 47, 48 and 49) which suggests this possibility, but the conditions in this experiment are not quite clear. A fragment of the iris was in this case (No. 39) transplanted into the cartilaginous capsule of the otic labyrinth. This fragment stimulated the formation of a lens (Fig. 49) from a strand of epithelium that had grown in from the edge of the wound. It is uncertain, however, whether in this case the "lentogenic" stimulus issued from the iris or perhaps from retinal cells contained in the transplanted fragment. For while on p. 429 Wachs states that this fragment was "ein Stück der oberen Iris" and that "nach 28 Tagen . . . war das Stück mit Goldpigment noch deutlich sichtbar," on p. 447 (in the summary) he refers to it as "ein Stück des Auges" and in the record (p. 423) this case (No. 39) is enumerated among those in which "ein Stück des oberen Augenteiles" was transplanted into the labyrinth. Wachs himself, however, does not state whether he assumes that in this case the regenerated epithelium formed the lens under the influence of retinal cells or of the iris.

There is no reason, however, why the latter possibility should be excluded. It is certainly not excluded by those experiments

of Wachs in which a fragment of the eye cup containing both iris and retina (cf. Fig. 50) transplanted "dicht unter die Haut" has failed to stimulate lens-formation from the latter, but instead has so altered it that it gave the appearance "einer kleinen Cornea." For in these cases the failure to induce the lentogenic reaction might have to be attributed to the retinal as well to the iris-part of the transplanted fragment; it may, however, be due to the circumstance that the skin of the relatively old larvae was no longer capable of responding to a "lentogenic stimulus." The latter possibility may be implied from the (above-mentioned) case (pp. 237 and 241) in which owing to the operation an ingrowing strand of epithelium formed a lens under the influence of the transplanted fragment of the optic cup. For, as Wachs himself concludes, we are in that case (No. 39) dealing with "Abkömmlinge von Hautzellen, die, neugebildet und noch undifferenziert, offenbar die gleiche Fähigkeit der Linsenfaserbildung haben können, wie einst die junge Haut . . . " (*l. c.*, p. 430).

But while there is, as we see, no definitely known instance of lens-formation owing to a stimulus from the iris, there is, on the other hand, nothing that would contradict the assumption that the iris is capable of exerting such a stimulus on ectodermal derivatives. Moreover, the very fact that the other parts of the optic cup (retina and tapetum nigrum) can furnish the lentogenic stimulus would make it appear very probable that the iris which genetically is also a part of the optic cup (proper), is likewise possessed of the ability to induce the "lentogenic reaction."

Granting the correctness of this assumption (which eventually may be borne out by suitable experiments), we would in the case of the iris be confronted by the same conditions as in the case of the retina. It is known that the iris can form a lens, and if, as seems probable, it can also induce the formation of a lens from ectodermal epithelium, the assumption may be justified that in the formation of a lens from the pupillary edge of the iris, which is an ectodermal derivative, its cells respond to their own "lentogenic enzyme."

These conditions apparently obtaining in the formation of lentoids from the retina and very probably also in the "regeneration" (secondary formation) of the lens from the iris are in prin-

ciple similar to the conditions obtaining in the primary (embryonic) formation of the lens, although the morphogenetic scheme of the latter cannot be fully applied to either the retinal lentoids or to the lens formed from the iris. The chief difference between the two modes of the morphogenesis of the lens is that while the origin of the "lentogenic stimulus" and the part responding to it are locally separate in the primary formation of the lens, they would—granting the correctness of our interpretation—coincide locally in the secondary formation of the lens from the iris or of a lentoid from the retina.

In order to fully understand the morphogenetic process underlying the "regeneration" of the lens from the iris (or of lentoids from the retina), it would also seem necessary to explain just how the "lentogenic stimulus," the latent capacity for which is probably possessed by the whole optic cup proper, is activated on the extraction of the primary lens. An answer to this query is, I believe, partly given by some of Fischel's ('02) experiments, which I shall review in the following.

The lens is so tightly enclosed by the iris that on its extraction slight injuries to the latter result. No matter how carefully the operation may be performed, slight, unnoticeable, lesions (abrasions?) of the cells of the pupillary edge of the iris may be unavoidable.[1] The significance of these, otherwise perhaps negligible, physical alterations of these cells of the iris resulting from the operation suggested itself to Fischel ('02, pp. 106–109 ff) from the observation of small lenses and lentoids formed not only by the pupillary edge but also from other parts of the iris. Being aware of accidental injuries to the eye cup and to the iris in some of his experiments he thought of a possible causal connection between the latter and such lenses or lentoids located in various parts of the iris. In order to test the correctness of his tentative interpretation he performed a number of experiments in which besides the exstirpation of the lens various parts of the iris were purposely injured. On microscopic examination of the results he was able to observe that small lenses or lentoids were formed

[1] According to E. Uhlenhuth (quoted from Loeb '16) who cultivated fragments of the iris by Harrison's explantation method, it is to these abrasions that the loss of the pigment by the cells of the iris is due. Owing to this injury of the cells the pigment granules are liberated into the lumen of the optic cup where, as Wolff ('95) has observed, they may be absorbed by leucocytes.

from just such intentionally, and still discernibly distorted, parts of the iris. In several experiments Fischel observed besides the formation of a lens from the pupillary edge of the iris small double lenses, or twin (fused) lenses and in one case even three lenses in other parts of the latter. For the formation of these double (or multiple) structures Fischel, I think, correctly accounts by close proximity to each other of two (or more) places of injury.[1] Even the size of such lens-like structures he regards as dependent upon the extent (number of cells) of the injured area.

The lens formed by the pupillary edge of the iris after exstirpation of the primary lens is usually as large and as well differentiated as the latter. This, according to Fischel, is due to the circumstance that on extraction of the lens a large area comes under the stimulus of the injury, and also to the fact that the pupillary margin of the iris being an epithelial fold, it affords the best means for the formation of a lens-bud (the "Knoten"—Wolff '95), the first step in which is always a folding of the corresponding part of the epithelium.

Another apparent confirmation of this "Reizhypothese" was furnished by the lentoids of the retina. The latter were observed by Fischel ('02) in many experiments in which owing to the extraction of the lens the eye bulb sustained accidental injuries. The experimental test—intentional injury of the retina—again gave positive results. The "retinal lentoids" could be demonstrated to be due to transformation of thus injured cells or groups of cells of the retina into such lens-like structures. The fact that only the latter and not fully differentiated lenses of large size can be formed by the retina on mechanical stimulation is, according to Fischel, accounted for by the small area of such stimulation and by the difficulty of forming a large fold.

This ability of the retinal cells to become transformed into lens-like structures Fischel regarded already in 1902 as no more surprising than, (as demonstrated by his experiments), that of any part of the iris, or of the epithelium of the skin in normal

[1] In some experiments of Wachs (*l. c.*) in which a fragment of the iris was introduced into the posterior eye chamber of another animal whose lens has been extracted, both the implanted fragment and the animal's own iris formed a lens. In some of these cases where the fragment coalesced with the iris, two more or less fused lenses were observed at the point of coalescence, one formed by the foreign fragment and one by the iris.

ontogeny. Already at that time he regarded it as a primary potency of all derivatives of the ectoderm. As the additional factor, however, necessary for this transformation of ectodermal derivatives he assumed an unknown stimulus which in the "regeneration" of the lens from the iris "*in jenen Alterationen zu suchen ist, welche das regenerierende Gewebe direkt durch den experimentellen Eingriff selbst erfährt* . . . von welchen die Zellen der Iris bei der Linsenextraktion betroffen werden" ('02, p. 106).

This unknown stimulus Fischel ('16) now regards as a chemical one from a secretion by the optic cup, or more specifically, from the retina which in conformance with Spemann and Wachs he also assumes. To the stimulus from this secretion he attributes the transformation of the supra-ocular epidermis into a lens in the embryo as well as the like transformation of the cells of any injured part of the optic cup (iris or retina).

While in the main I agree with Fischel by recognizing the necessity of an injury to that part of the iris or retina from which the secondary lens or lentoid arises, as proven, I am, as already stated, inclined to doubt the probability of a secretion by the eye cup as the additional factor involved in the stimulus for the formation of the secondary lens. Undoubtedly, with the present data at hand, it is difficult to deny that some chemical process is apparent in the morphogenesis of both the primary and the secondary lens. This process, however, may, as was pointed out in the preceding pages, be due to a substance (a "lentogenic enzyme") apparently contained by all parts of the eye cup already in the stage of the optic vesicle or even earlier. The activation, however, of this "enzyme" seems in the case of the "regeneration" of the lens to be in some way due to the injury of those cells of the iris which subsequently become transformed into lens fibers.

What would yet remain to be explained is the nature of the relation of the injury of the cells to the activation of their own "lentogenic enzyme." On this point, however, I should venture no opinion at the present time.

One apparent objection to our interpretation may yet be considered.

It is known from experiments by Wolff ('03) that if a part of

the iris be removed by cutting (iridectomy) without removing the lens, the iris will regenerate but form no lens-like structures. Wachs (*l. c.*) attempted to account for this fact by the assumption of a secretion from the lens which would inhibit the formation of another lens. The results of some of his experiments, he believes, substantiate this conclusion. In these experiments the lens was removed and subsequently the lens of another animal of the same or a related species was introduced into the eye. In no case in which the foreign lens healed into the eye was a lens "regenerated." This result, however, permits of another interpretation, in which no resort is made to the improbable assumption of the "antisecretion" by the lens.

Indeed, Wachs's own ingenious experiments performed for the solution of this particular part of the lens-problem would seem to disprove rather than to prove the assumption of a secretion. For they show that the "regeneration" of a lens is inhibited only when the (smaller) lens implanted into the eye, heals into it, *i. e.*, comes into close contact with the iris ("so dass die verengerte Iris sich ihr ringsum dicht anschliesst . . . ," p. 404). If, however, such contact is not effected—"liegt jedoch die aus dem jüngeren Tiere implantierte kleinere Linse mehr oder weniger in der vorderen oder hinteren Kammer, so w'rd eine Regeneration eingeleitet."

Wachs has ascertained beyond doubt that the inhibition is in these cases due not only to the mechanical effect of the contact, but very apparently also to a chemical action. While, however, he strongly inclines to the belief that the chemical action is due to a secretion, I think it can easily be shown also in all other of his experiments by which the matter was tested, that this chemical action is conditioned by contact of the implanted lens with the iris.

These important experiments of Wachs, strengthened further by some interesting results of Fischel's ('02) experiments[1] would

[1] Fischel exstirpated the lens and replaced it by small spherical fragments of the potato tuber. Whenever this "imitation lens" was large enough to fit the pupilla, no "regeneration" of a lens from the latter took place. If, however, its diameter was smaller than that of the pupilla, partial "regeneration" of the lens or at least an "attempt" towards it was noted, complete "regeneration" being impossible owing to mechanical obstruction by the potato fragment. This experiment clearly demonstrates that the extraction of the lens eliminates not an indifferent body, but one with a specific, *inhibiting*, action.

seem, however, to leave no doubt that the lens contains something, some substance that apparently inhibits the formation of another lens from the iris which would invariably occur in its absence.[1] This substance, however, again need not necessarily be secreted, in order to neutralize the action of the "lentogenic enzyme." Just what the nature of this substance may be, is, of course, altogether a matter of conjecture. It might perhaps be imagined as an "antibody" ("antigen") contained in the peripheral part (the epithelial cells) of the lens and the realization of its inhibiting action may be due to close contact of that part of the lens with the epithelium of the iris.

This "antibody" being, just like the lens, a product of the "lentogenic reaction," we may perhaps in the case of the vertebrate eye eventually have a striking example of some of the factors (synthetic enzyme action—Loeb, '16) concerned not only with development and growth, but also with the limitation of the latter[2] for the attainment of proper size relations, and with the maintenance of a chemical equilibrium indispensible for the undisturbed existence of a structure or an organ.[3]

[1] Fischel ('16) has recently furnished another interesting example of the apparent chemical action of the lens which he also considers as a "secretion." He transplanted in larvae (about 3 cm. large) of *Salamandra maculosa* the exstirpated lens under the skin of various parts of the head or trunk. As a result he observed that, while the lens underwent a gradual dedifferentiation and eventual absorption, the skin above the transplantate showed remarkable changes. The unicellular glands (the cells of Leydig) disappeared from the corresponding region of the skin, which, owing to the morphological changes, by its appearance suggested a similarity to "frühe Entwicklungsstadien des Hautepithels" (p. 37). Eventually this region of the skin became transparent and was very similar to a fully differentiated cornea.—Similar observations were, as mentioned above (p. 242), recorded also by Wachs (*l. c.*) on transplanting parts of a fully differentiated optic cup under the skin.

[2] The fact that the "regenerating" lens does not grow indefinitely (as it might, if it were a benign tumor—regarded as such at one time by Fischel, '00), but is limited in its growth to attain just the size to fit the pupilla, has recently occasioned J. Loeb ('16) to raise the question regarding the factors limiting growth. According to our assumption it might perhaps be imagined that every tissue (or structure) elaborates during its development a substance (an "antibody") which inhibits its growth beyond certain limits. In this case both growth and its automatic limitation would be conditioned by the same factors of development.

[3] Fischel ('16) concludes from his experiments that the already fully differentiated lens needs the chemical influence of the eye for its normal (undisturbed) existence.

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